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CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Commissioner for Patents, Washington, D.C. 20231, on the 17th day of September, 2002.

Konstantinos Andrikopoulos

Konstantinos Andrikopoulos, Reg. No. 48,915

COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

Sir:

DECLARATION OF MATTHEWS O. BRADLEY UNDER 37 C.F.R. §1.132

I, Matthews O. Bradley, state and declare the following:

1. I am a co-inventor in the above-identified patent application. I make this Declaration in support of an Amendment filed in connection with the above-identified patent application.

2. This Declaration details experiments that were carried out under my direct supervision and control. All experiments involved the *in vivo* contacting of tumors with: (i) *anticancer agents*, and/or (ii) *anticancer agent-fatty acid conjugates*.

3. The experiments described herein were designed to specifically compare the drugs described in ¶2 above with respect to: (i) their antitumor effects; (ii) their dosage toxicity effects (Maximum Tolerated Dose – MTD); and (iii) their pharmacokinetic properties.

4. The materials and methods utilized in the experiments described herein were essentially the same as those taught in the specification of the instant application. Briefly, an *anticancer agent-fatty acid conjugate* and *anticancer agent* alone were independently administered to an established animal tumor model frequently utilized by those of skill in the art [for example, *docosahexanoic acid (DHA)-paclitaxel conjugate* and *paclitaxel* alone were independently administered in mice bearing M109 lung tumors].

5. As will be seen from ¶6-¶9 detailing the experiments below, the following unexpected findings were observed: (i) the *anticancer agent-fatty acid conjugate* possesses increased antitumor activity relative to

the *anticancer agent* alone; and (ii) the *anticancer agent-fatty acid conjugate* is less toxic than the *anticancer agent*.

6. In one experiment, both *paclitaxel* and *DHA-paclitaxel* were formulated in 10% Cremophor EL-P/10% ethanol/80% normal saline, and independently administered *i.v.* in mice bearing M109 lung tumors. *Paclitaxel* at its MTD of 20 mg/kg (optimum dose) caused neither complete nor partial regressions in any of 10 mice bearing M109 tumors. By contrast, *DHA-paclitaxel* caused complete regressions that were sustained for 60 days in 4 of 10 mice at 60 mg/kg, 9 of 10 mice at 90 mg/kg, and 10 of 10 mice at the *DHA-paclitaxel* MTD of 120 mg/kg. Therefore, *DHA-paclitaxel* possesses increased antitumor activity, and can be given at 4.4 times the *paclitaxel* MTD (in molar terms) (i.e., it is less toxic than *paclitaxel).*

7. In one experiment, both *paclitaxel* and *DHA-paclitaxel* were formulated and administered to mice bearing M109 lung tumors as described in ¶6. *Paclitaxel* at its MTD of 20 mg/kg (which is equimolar to the *DHA-paclitaxel* dose of 27.4 mg/kg) caused neither complete nor partial regressions in any of 10 mice bearing M109 tumors. By contrast, *DHA-paclitaxel* caused complete regression in 1 of 10 mice (the least detectable increase in cures) at 36 mg/kg, well below its MTD of 120 mg/kg. Therefore, the lowest MTD increase for *DHA-paclitaxel* that results in an improved cure rate relative to *paclitaxel* is 31% {[36 mg/kg – 27.4 mg/kg]/ [27.4 mg/kg]}.

8. In one experiment, both *paclitaxel* and *DHA-paclitaxel* were formulated as described in ¶6. *Paclitaxel* and *DHA-paclitaxel* were independently administered to mice bearing M109 lung tumors in single *i.v.* doses, at either equimolar or equitoxic concentrations, and the tumor uptake of each drug was measured. Examining the area under the drug concentration-time curve (AUC) for both *paclitaxel* and *DHA-paclitaxel* in tumors, revealed that *DHA-paclitaxel*'s AUC is 8-fold higher than *paclitaxel*'s at equimolar doses, and 61-fold higher at equitoxic doses. These data, therefore, are indicative of the higher *DHA-paclitaxel* uptake by tumors than that for *paclitaxel*.

9. In one experiment, both *paclitaxel* and *DHA-paclitaxel* were formulated as described in ¶6. *Paclitaxel* and *DHA-paclitaxel* were independently administered to mice bearing M109 lung tumors in single *i.v.* doses, at equitoxic concentrations (at each drug's MTD), and the tumor uptake of *paclitaxel* was measured. Examining the area under the drug concentration-time curve (AUC) for *paclitaxel* in tumors derived from either *i.v. DHA-paclitaxel* or *i.v. paclitaxel,* revealed that the tumor AUC of *paclitaxel* derived from *i.v. DHA-paclitaxel* is 6-fold higher than for *paclitaxel* derived from *i.v. paclitaxel.* In the same experiment it was also revealed that the time the *paclitaxel* concentration in tumors is above the minimum therapeutic concentration required to halt tumor growth is 10-times longer following *DHA-paclitaxel* administration than following *paclitaxel* administration. Therefore, these observations further explain the increased antitumor efficacy of the *anticancer agent-fatty acid conjugate* relative to the *anticancer agent*.

10. Similar results to those described in ¶6-¶7 above (for mice bearing M109 lung tumors) were obtained using mice bearing HT-29 colon carcinoma tumors.

11. In Phase I/II clinical trials using human patients enrolled in at least thirty centers throughout the United States, the United Kingdom, Germany, and the Netherlands, treating eight different types of cancer, it was discovered, unexpectedly, that *DHA-paclitaxel* can be safely administered at doses that are 3.3-5.9 times (on a molar basis) the doses of *paclitaxel* that are regarded as safe in the clinic (i.e., the *paclitaxel* MTD). Also unexpected has been the finding that even at the higher *DHA-paclitaxel* doses that are being used, several key toxicities are reduced compared to those that occur with the administration of *paclitaxel* at lower doses. For example, *DHA-paclitaxel* hypersensitivity reactions occur at 16% of the rate observed

for *paclitaxel*, hair loss (alopecia) occurs at 5% of the rate observed for *paclitaxel*, nerve toxicity (peripheral neuropathy) occurs at 38% of the rate observed for *paclitaxel*, and damage to the mucus membranes of the body (stomatitis/mucositis) occurs at 51% of the rate observed for *paclitaxel*.

12. In Phase I/II clinical trials using human patients enrolled in at least thirty centers throughout the United States, the United Kingdom, Germany, and the Netherlands, treating eight different types of cancer, it was discovered, unexpectedly, that *DHA-paclitaxel* can be safely administered at doses that are 6.7-8.1 times (on a molar basis) the doses of *Taxotere*® (only other FDA-approved taxane drug) that are regarded as safe in the clinic (i.e., the *Taxotere*® MTD). Also unexpected has been the finding that even at the higher *DHA-paclitaxel* doses that are being used, several key toxicities are reduced compared to those that occur with the administration of *Taxotere*® at lower doses. For example, *DHA-paclitaxel* hypersensitivity reactions occur at 44% of the rate observed for *Taxotere*®, hair loss (alopecia) occurs at 6% of the rate observed for *Taxotere*®, nerve toxicity (peripheral neuropathy) occurs at 46% of the rate observed for *Taxotere*®, and damage to the mucus membranes of the body (stomatitis/mucositis) occurs at 38% of the rate observed for *Taxotere*®.

13. Further *in vivo* and *in vitro* experiments confirming the foregoing findings, as well as experimental details for all of the experiments described herein, can be found in two of my recent publications (Bradley M.O. et al., *Clin. Cancer Res.*, 2001, 7:3229-3238; Bradley M.O. et al., *J. Controlled Release*, 2001, 74:233-236). These two publications are submitted concurrently herewith as Exhibits 1 and 2 respectively.

14. The finding that the *anticancer agent-fatty acid conjugate* is less toxic than the *anticancer agent* itself allowing administration of much higher (molar) doses of the *conjugate* is unexpected.

15. The finding that the *anticancer agent-fatty acid conjugate* remains in tumors for long times at high concentrations is unexpected.

16. The finding that the *anticancer agent-fatty acid conjugate* possesses increased antitumor activity relative to the *anticancer agent* is unexpected.

17. The finding that the *anticancer agent-fatty acid conjugate*, which has much lower toxicity, achieves actual cures at only about 30% higher than the MTD for the *anticancer agent* alone (on a molar basis), also is unexpected.

18. In view of the foregoing unexpected findings it is my opinion that the compositions and methods of the above-identified patent application as currently claimed in the above-identified Amendment would not be obvious to one of ordinary skill in the art in view of claims 1-6 of U.S. Patent 5,919,815.

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this document and any patent which may issue from the above-identified patent application.

Date: 9-9-02

Matthews O. Bradley, Ph.D.

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